

CHARACTERIZATION OF THE OSTEOTOMY CALLUS USING FT-IRI IN A RABBIT ULNAR MODEL

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**Introduction**

Natural course of fracture healing includes: (i) interfragmentary stabilization by periosteal and endosteal callus formation, (ii) fibrocartilage differentiation and (iii) substitution of avascular and necrotic areas by haversian remodeling. This type of healing is often referred as secondary healing. Radiologic characteristics are more or less abundant callus formation, temporary widening of the fracture gap by osteoclastic resorption and a slow disappearance of the radiolucent line due to fibrocartilage mineralization and bone formation.

On the other hand, direct or primary bone healing secondary to anatomic reduction and stable fixation. Lack of external callus formation and gradual disappearance of the narrow of the fracture line is the main criteria.

The process of mineralization is different in the two different scenarios due to the inherent rigidity provided by plate fixation when compared to the natural biologic process of bone healing.

FT-IRI has the capacity to measure the quality of lamellar bone, woven bone and calcified cartilage; evaluating different parameters that include: amount of mineral, amount of organic matrix, Mineral/matrix ratio, orientation of appatite and collagen, quality of bone defined as crystallinity/maturity ratio, amount of type B carbonate and maturity of collagen.

In order to compare the two different process of bone mineralization FT-IRI was applied to the following animal model. It is hypothesized that the degree of rigidity during fracture repair will alter the mineral properties of the fracture callus.

**Methods**

A rabbit ulnar model similar to that previously described by Bostrom et al. was used. Forty-two Male New Zealand white rabbits (6 moths old - 4.0-5.0 kg) were used in an IACUC approved study by the Institutional Animal Care and Use Committee.

After general anesthesia was provided, each rabbit's right and left forelimb was shaved and prepared with povidine and alcohol solution, and then sterilely draped. A longitudinal incision was made over the mid-portion of the ulna and the mid-ulnar diaphysis with extra-periosteal exposure.

82 limbs were available for the study; each forelimb was randomly designated to one of the following groups (21 in each group):

1. 1mm osteotomy 3 cm proximal to the ulnocarpal joint fixated with compression plate applied using AO technique.
2. 1mm osteotomy 3 cm proximal to the ulnocarpal joint fixed without compression
3. 1mm osteotomy with no fixation.
4. 3mm osteotomy with no fixation

The osteotomy was made using a 1 mm high speed dental burr. After surgery the soft tissues were closed in layers. Animals were euthanized Euthanasia was performed with an overdose of intravenous Phenobarbital at the following timelines, 2, weeks, 4, weeks and 8 wks postweeks after surgery.

Fourier Transform Infrared Microspectrometry (FT-IRI) was used to determine: (i) the relative amount of mineral present (ratio of integrated v1,v3 phosphate to Amide I contours, (ii) carbonate/phosphate ratio (integrated areas carbonate(v2) and phosphate peaks), and (iii) crystallinity (peak height ratio of subbands at 1030cm<sup>-1</sup>/ 1020cm<sup>-1</sup> peak). Bone specimens for FTIR were fixed in 90% ethanol, embedded in PMMA and cut into longitudinal 2µm sections using a Jung Model K Microtome (Hedelberg, Germany) as described elsewhere. Sections were placed onto BaF2 spectral windows for analysis. Imaging spectra were collected on a Perkin-Elmer Spotlight Spectrometer at resolution of 8cm-1 in transmission mode.

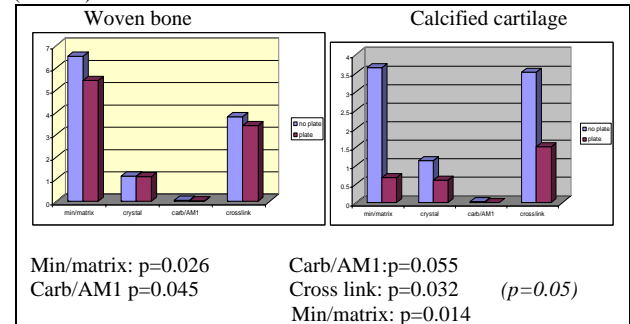
**Statistics**

ANOVA with p< 0.05 taken as significant was use between all groups. Student's t-Test for significance between the plated and non plated groups was used. Difference was consistent significant at (p<0.05)

**Results**

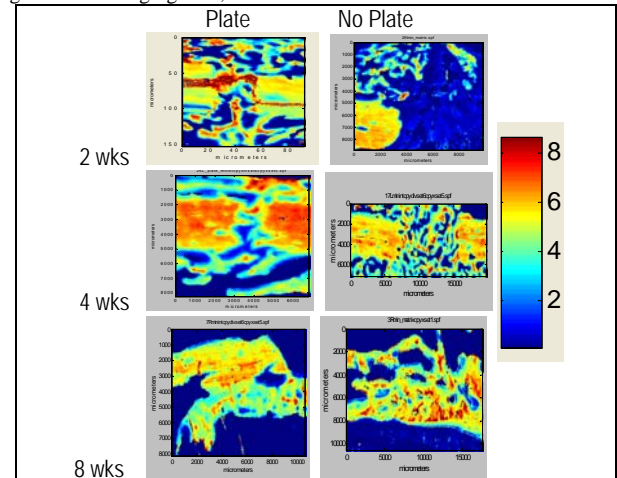
**2 weeks:** Mineral to matrix ratio and the carbon-Amine ratio was higher in the woven bone; also the carbon amine ratio was also higher in the non plated group compared to the plated group. When comparing woven bone between groups, the non-plated group demonstrated a more primitive less mature woven bone.

(Table 1). 2 weeks results



**4 weeks:** There was no statistical difference between woven bone and cortical bone but still a statistical difference was found in the amount of calcified cartilage.

Fig.1 FTI-IR imaging at 2, 4 and 8 weeks



**8 weeks:** a statistical difference mineral quality was not found between groups.

**Conclusions**

The previous data suggests that plate fixation accelerates the process of mineral development since the FT-IRI analysis demonstrated that during the early process of osteotomy healing (2 weeks) the amount of immature bone matrix and calcified cartilage was higher in the non plated osteotomy when compared to plate fixation; at four weeks, only the amount of calcified cartilage was statistically significant. At 8 weeks the mineral quality was equally comparable in both groups. Plate fixation accelerates mineralization apposition and crystal maturation in the early stages of healing over no fixation but the amount of mineral is equally comparable in both groups after the bone has healed at 8 weeks. This study, demonstrates that fracture fixation not only affects connective tissue sequence but also the quality of the tissue.

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